I N B I F O

I. Objectives - Defensive Research PM USA

II. Position of INBIFO Within Defensive Research

III. Why do we need INBIFO?

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IVA. Overview - Timetable of Significant Events

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- A. Types of Studies
- B. Capacity

VI. Budget

- A. Past - Present - Future
- B. Reasons For Decrease in 1978 Budget
- C. Distribution - PM USA/FTR/III Party

VII. Personnel

- A. Past - Present - Future

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X. Pharmacology

XI. In Vitro Assays - Ames Test

XII. What Have We Learned?

XIII. The Future

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

I. OBJECTIVES

A. Anticipate types, directions and extent of pressures relative to smoking and health.

B. Assure safety of new product components including additives.

C. Maintain experimental capability to test for appropriate biological effect in any situation.

D. Develop a battery of short term biological predictors of long term biological effects.

E. Advise top management of smoking and health strategy and tactics.

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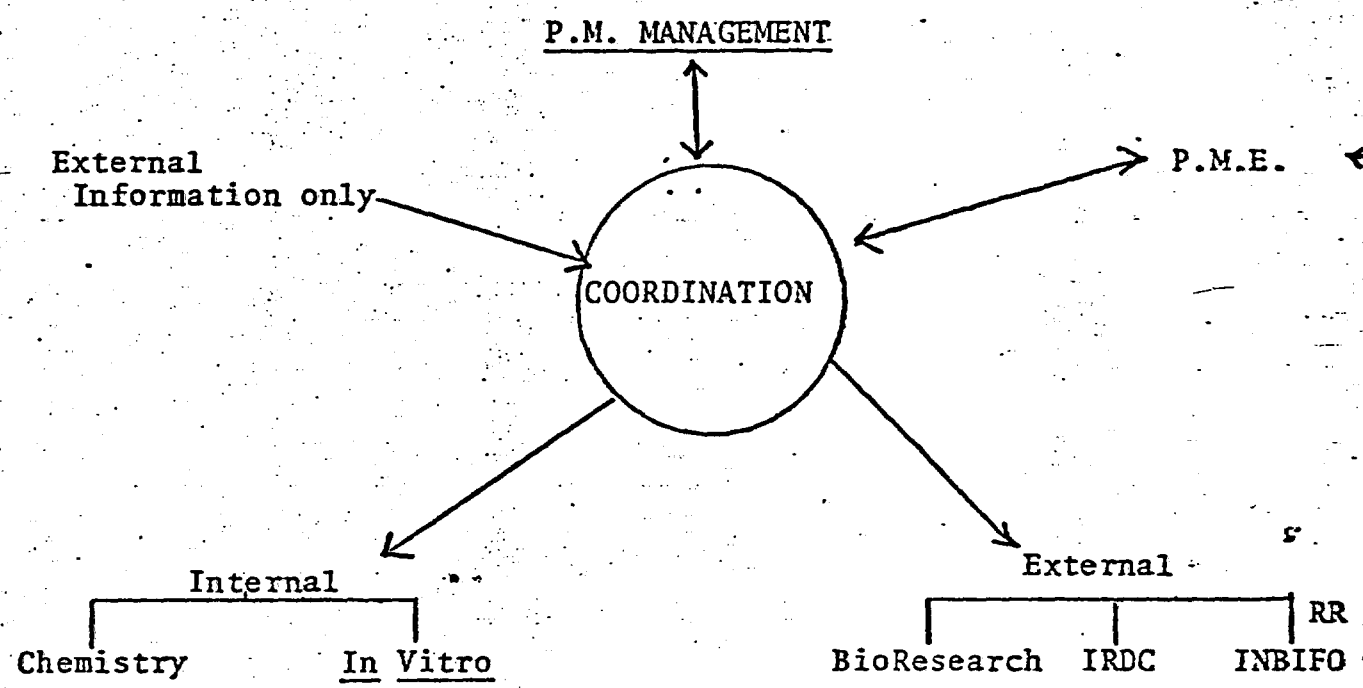
Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

II. POSITION OF INBIFO WITHIN DEFENSIVE RESEARCH



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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

III. Why do we need INBIFO?

A. Decrease in available domestic contract laboratories.

1. Several labs have folded - Industrial Bio-Test.

2. Regulatory Agencies requiring more and more product testing.

EPA Consumer Product Safety Commission

TOSCA

NIOSH

B. Long lead time required to initiate studies in contract labs.

1. Hazleton Laboratories - Altosid inhalation study ($20,000) - 30 day inhalation study required 9 months to complete.

2. Desired animal strain not always available. One parent of CAF_1 strain being purchased in large quantity by unknown sources.

C. Security

1. New additives and process variables can be tested with small probability of security leak.

2. Maintenance of confidentiality of additive identity difficult with contract laboratories.

D. Control

1. Mix of bioassays and priorities can be tailored to PM USA needs.

2. Experiments can be terminated at will as required without delay.

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Why do we need INBIFO? (continued)

E. Additive Testing

Hunter Committee - possible extension to cigarette paramete

French Additive Listing.

F. FTR and PME needs.

G. Response Capability with a Battery of Bioassays.

No adequate facilities in U.S. for long-term inhalation bioassay.

H. Maintain Competitive Stance.

Other companies doing long-term testing

L&M Patent

Additives Testing

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

IV. Time Chart - Acquisition of Technology

1967-73 Development Smoking machines for inhalation exposure

Inhalation exposure chambers

Animal containment tubes

SPF inhalation facility

14-Day inhalation study - rats

60-90-Day inhalation study - rats and mice

1973-75 2-Year Rat Inhalation Study - Expanded tobacco vs. non-expanded tobacco

1st Mouse Skin Painting Study - Reproducibility/ complete tumorigenesis

1976 2nd Mouse Skin Painting Study - Complete tumorigenesis/ promotion

90 and 120-Day Inhalation Study - Rats - low delivery cigts. ± menthol

Particulate Phase Deposition Studies with DCBP Labeled Smoke

1977 Laryngeal Epithelial Hyperplasia Studies - Rat

Developed Methodology for Walton-Type Exposure - Rats

Developed Methodology for Acute Inhalation Toxicity - Mice

Nicotine Pharmacology - LD_{50}, ED_{50}, Frog Posture, Isolated Guinea Pig Ileum, Rat Phrenic Nerve Diaphragm

Acquired Free Lung Cell Technology

Acquired Cell Culture Technology

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

1978 Developed Methodology for Chronic Hamster Inhalation Study

Completion of Laryngeal Hyperplasia Studies in Rat

21-Day Inhalation Study - Rat - New End Points

5th Mouse Skin Painting Study - Cocarcinogenesis Study

Nicotine Pharmacology - BP Rat, Methodology Improvements being Acquisition of Ames Test

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

IVA. Overview - Timetable of Significant Events

AMMO FOR TIMETABLE

TO BE SELECTED

1967	Oberländer Uffer	Developed 1st Inhalation Exposure Chamb Developed Animal Contaminant Tubes. Improved Syringe Smoking Machine.
1968	Oberländer Uffer	Further development of Inhalation Chamb Inside/outside chamber with positive pressure. Increase capacity from 30 to 120 rats - heat exhange not solved - chamber design abandoned. FTR Smoking Machine modification - jet pump changed from glass to metal with reduced loss of particulate phase.
1969	Oberländer Uffer	2 Air jet smoking machines to PM (one for CTR, one for ET evaluation at Robins).. Automatic cigarette feeding device developed for Borgwaldt machine.
1970	Oberländer Uffer	1002974056
1971	Oberländer Uffer	Compressed air cigarette injection developed. INBIFO acquired.
1972	Oberländer Uffer	Development begins on INBIFO positive

Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

		pressure automatic smoking machine. Development begins on INBIFO tape SPF. Inhalation Facility for Porz with Marienburg as a Pilot Facility. Battelle smoking machines (2) Rat - goblet cell study Hamster - DENA intratracheal injecti
1973	Oberländer Uffer Marienburg	February - INBIFO acquires land in Por New animal containment tubes with SS he holders developed. November - 2-year comparative inhalatio study on rats with ET and control cigar begins.
1974	Oberländer Uffer Marienburg Porz	Porz occupied in late 1974. Skin painting-Begin 1st mouse skin painting study (1R1).
1975	Marienburg Porz	60,90 and 120 day inhalation study on r 5 and 7 application days/week. (Method development - Interim Dissectio June - termination of 2 year inhalation begin recovery. Immunology-free lung cells - no differe net vs. ET.

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

1976	Porz	Acute inhalation on rats - determine maximum TPM load.
		90-day and 120-day inhalation on rats: 2 test cigarettes and control F2=W3=2R1F on equal TPM basis (F_2=W_3) <2R1F on per cigt. basis.
		1st Laryngeal Epithelium study.
		Particulate phase deposition with DCBP labeled smoke.
		42-day continuous vs. intermittent smoke exposure on rats.
		Begin 2nd skin painting study (1R1, 2R1, AKZ, ALB).
1977	Porz	Inhalation study - varied TPM and gas phase rats
		21-day inhalation study, rats. reproducibility study. Laryngeal Epithelial Hyperplasia
		21-Day inhalation study with added acetaldehyde - rats.
		Food deprivation study - thymic and splenic atrophy - rats.
		21-day inhalation study with added NO - rat
		21-day inhalation study with NO and NO_2 - rats.
		21-day inhalation study net vs. 2R1 - differences between previous studies disappeared.
		42-day inhalation study whole smoke and gas phase - rats.
		Puff exposure study - 2-week-rats- Walton type study methodology available.
		7-day inhalation study - hamsters vs rat - Laryngeal hyperplasia NSD.
		21-day inhalation study - 2R1, ALB, AKZ, AKJ - Laryngeal Hyperplasia NSD - dose dependent TPM effect.

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Acute inhalation toxicity - mice.

Mouse skin painting DMBA no effect level.

Third skin painting 2R1 and low tar reference.

Fourth skin painting 2R1 + endotoxin treatment.

Nicotine pharmacology LD_{50} and ED_{50} (convulsion) mice - IV.

Nicotine and nicotine derivatives pharmacology ED_{50} - frog posture.

Nicotine and derivatives/analogue pharmacology-isolated guinea pig ileum. Rat Phrenic Nerve - diaphragm (nicotine relative to tubocurarine inhibition)

1978 Porz

21-day inhalation on rats NET & 2R1 with added NO-Laryngeal Hyperplasia and free lung cells (NO decreased number of PMN).

14-day inhalation rats daily dissection - acute end points and time response Laryngeal Hyperplasia.

Puff exposure study with DCBP and 2,4-D-Walton-type exposure - DCBP better tracer than 2,4-D.

Food deprivation study with rats + nicotine injection in progress.

Intermittent vs. continuous exposure hamsters with felt collars - prediction of long term exposure conditions - methodology available.

42-day inhalation - rats - 2R1, low alkaloid, LTF IIIA and LTF IIJF - Laryngeal Hyperplasia dose dependent TPM effect.

21-day inhalation study - rats - new end points - in progress.

Fifth skin painting study - cocarcinogenesis study with RL and tobacco blend.

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Nicotine pharmacology - blood pressure anesthetized rat.

Nicotine pharmacology - synthetic compounds.

Nicotine pharmacology - isolated guinea pig auricle.

Nicotine pharmacology - BP, threshold, guinea pig - ileum and auricle - methodology improvement.

Nicotine pharmacology - synthetic compounds.

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

V. RESPONSE CAPABILITY AT INBIFO

Types of Studies

Acute inhalation - mouse and hamster

Subchronic inhalation - rat and hamster

Chronic inhalation - rat and hamster

Mouse skin painting - complete, initiation, promotion, cocarcinogenesis.

Pharmacology - rat, guinea pig, frog, rabbit

General toxicology - mouse, rat, hamster

Pesticide analyses for FTR

Capacity

4 Automatic positive pressure smoking machines in 1 SPF laboratory - 600 rats, hamsters or mice.

3 Automatic positive pressure smoking machines in 1 SPF laboratory - 600 rats, hamsters or mice.

1 Mobile automatic positive pressure smoking machine.

1 Batelle piston smoking machine for acute inhalation in mice.

2 Batelle smoking machines for rodents

Each 72 animals - now being used for VdC

- previously FTR

- acute cytotoxicity suspension culture

7 INBIFO piston smoking machines - condensate prep.

2 Borgwaldt piston smoking machines (4 ports)

Analytical smoking

Acute cytotoxicity - monolayer cell culture

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Mouse skin painting 5,000 - 8,000 mice

Dependent on people and 3-day vs. 5-day/week schedule

Pharmacology of nicotine derivatives and/or analogues

30 compounds simultaneous with methods development.

Acute inhalation laboratory

Aerosol generators

spray nozzle

ultrasonic

evaporation

dust feeder

commercial aerosol propellant

2 Data General 32K computers

Pesticide analysis - gas chromatography

Chemiluminescence

Infrared spectrophotometers

Electronic and optical instruments for particle size determinations

1002974062

Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

BUDGET

PMUSA	ACTUALLY SPENT $	FTR
1967	3,874	
1968	42,336	
1969	78,538	
1970	26,622	
1971	79,050	
1972	252,092	
1973	658,713	
1974	973,941	
1975	1,695,447	
1976	2,130,785	
1977	2,735,143	
1978	2,737,301	1,154,286
1979	3,059,471	1,493,280
1980	3,015,256	1,301,169

	BUDGETED			
1981	4,030,000 (A)	ACTUAL: 3,479,000 (71%)	1,740,000	(A) 1,270,000 (26%)
1982	~~4,770,000~~ 4,160,000		~~2,100,000~~	1,680,000
1983	5,820,000		2,500,000	
1984	6,490,000		2,800,000	
1985	7,030,000		3,000,000	

3D PARTY

166,000 $ (3%)

(A) Invoiced as of April 30,1981

PMUSA 1,114,000 (below budget 196,000)

FTR 404,000 (" " 166,000)

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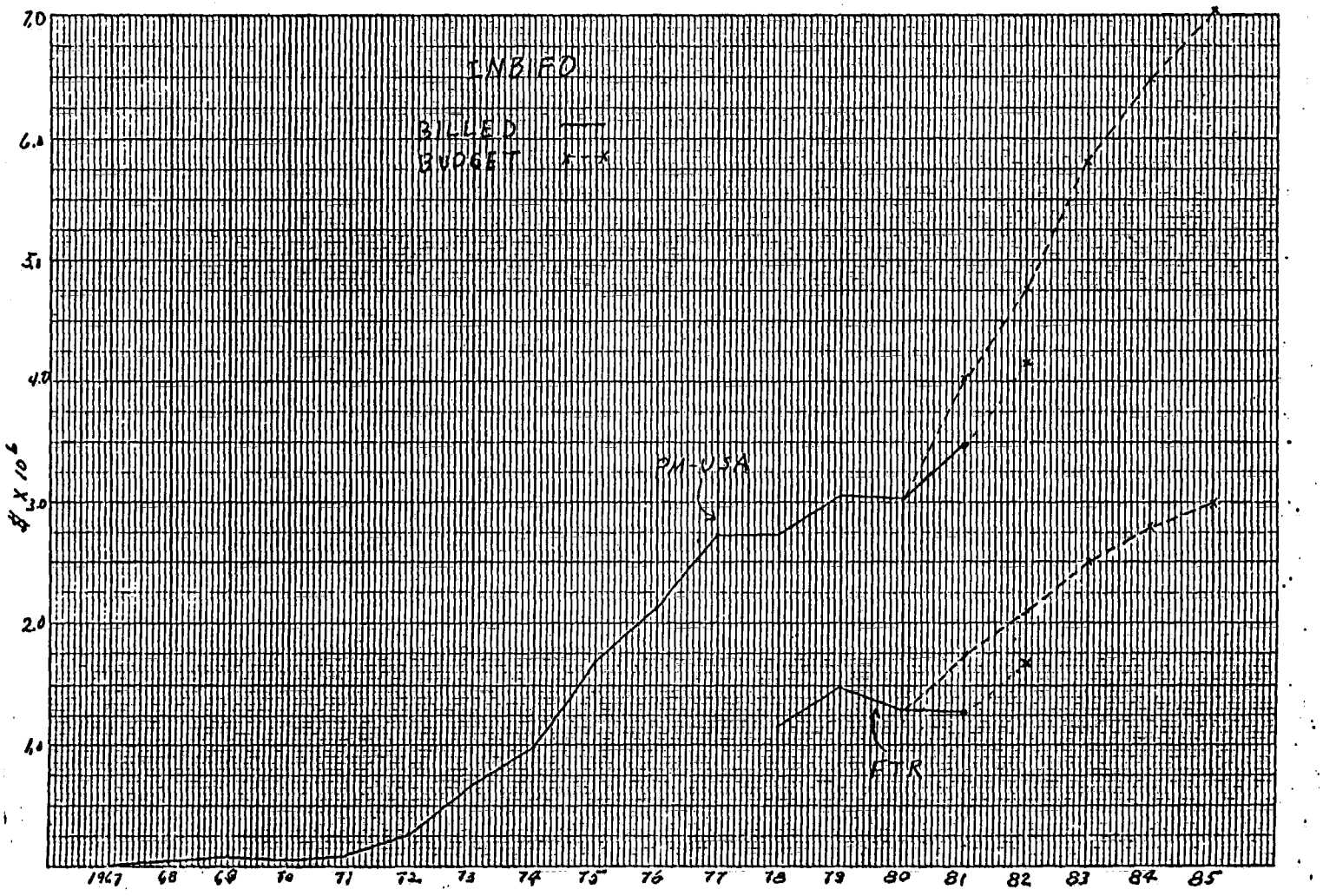
Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

K+E 10 X 10 TO ½ INCH 7 X 10 INCHES KEUFFEL & ESSER CO. MADE IN U.S.A.

46 1320



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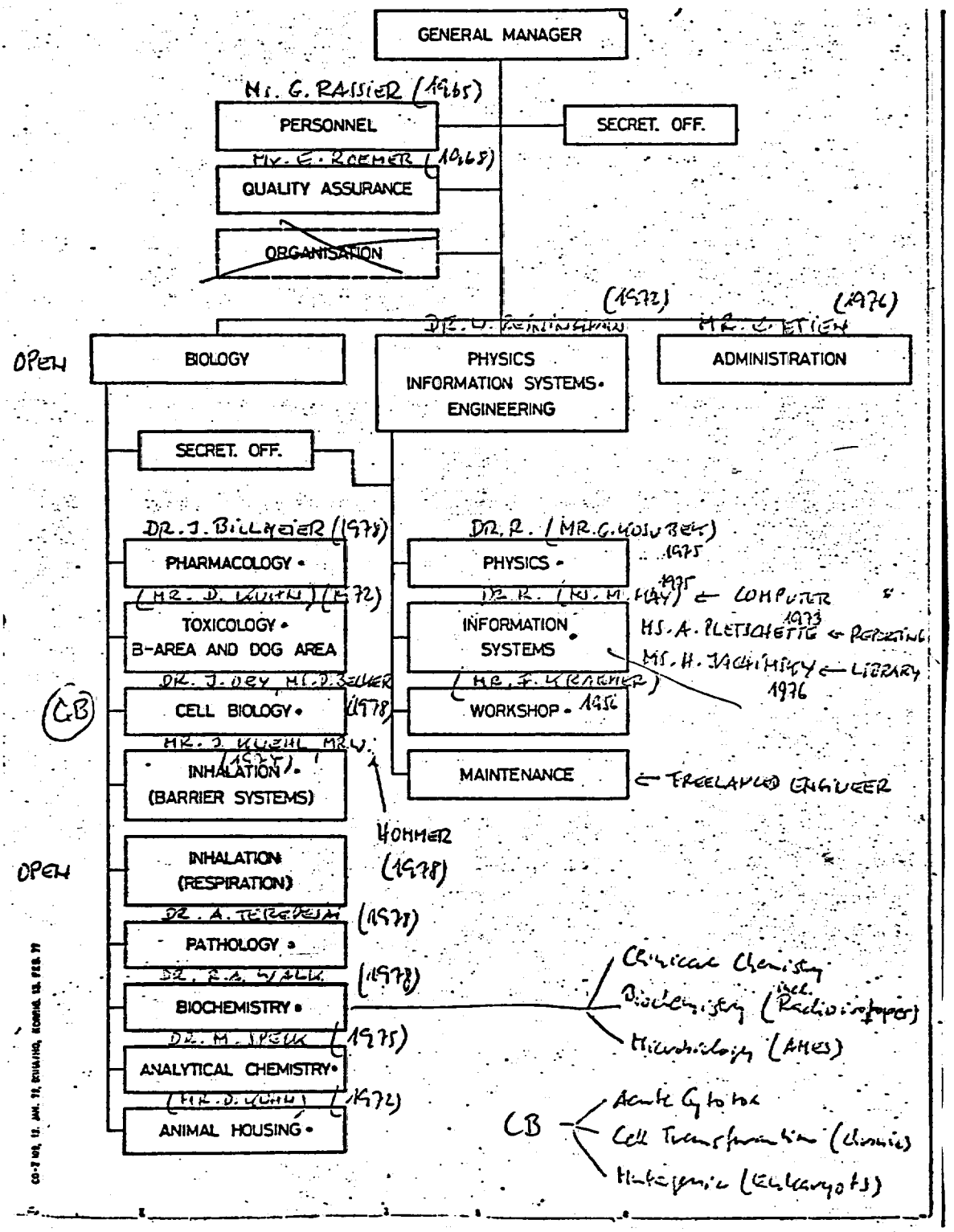

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

PERSONNEL

YEAR	NUMBER		
1967	24	average	
1968	21		
1969	28		
1970	34		
1971	29	end of year	
1972	44		
1973	52		
1974	67		
1975	69		
1976	72		
1977	89		
1978	111		
1979	117		
1980	114		
1981	119 as of May 27, 1981	→	126
1982	145	budgeted	135
1983	150		140
1984	155		145
1985	160		150
			155

PROFESSIONALS

End of 1978	11
End of 1980	16
May, 1981	18
June, 1981	19
End of 1981	21
1982	26 Budgeted 23 as of May 82

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

K+E 10 X 10 TO ½ INCH 7 X 10 INCHES KEUFFEL & ESSER CO. MADE IN U.S.A.

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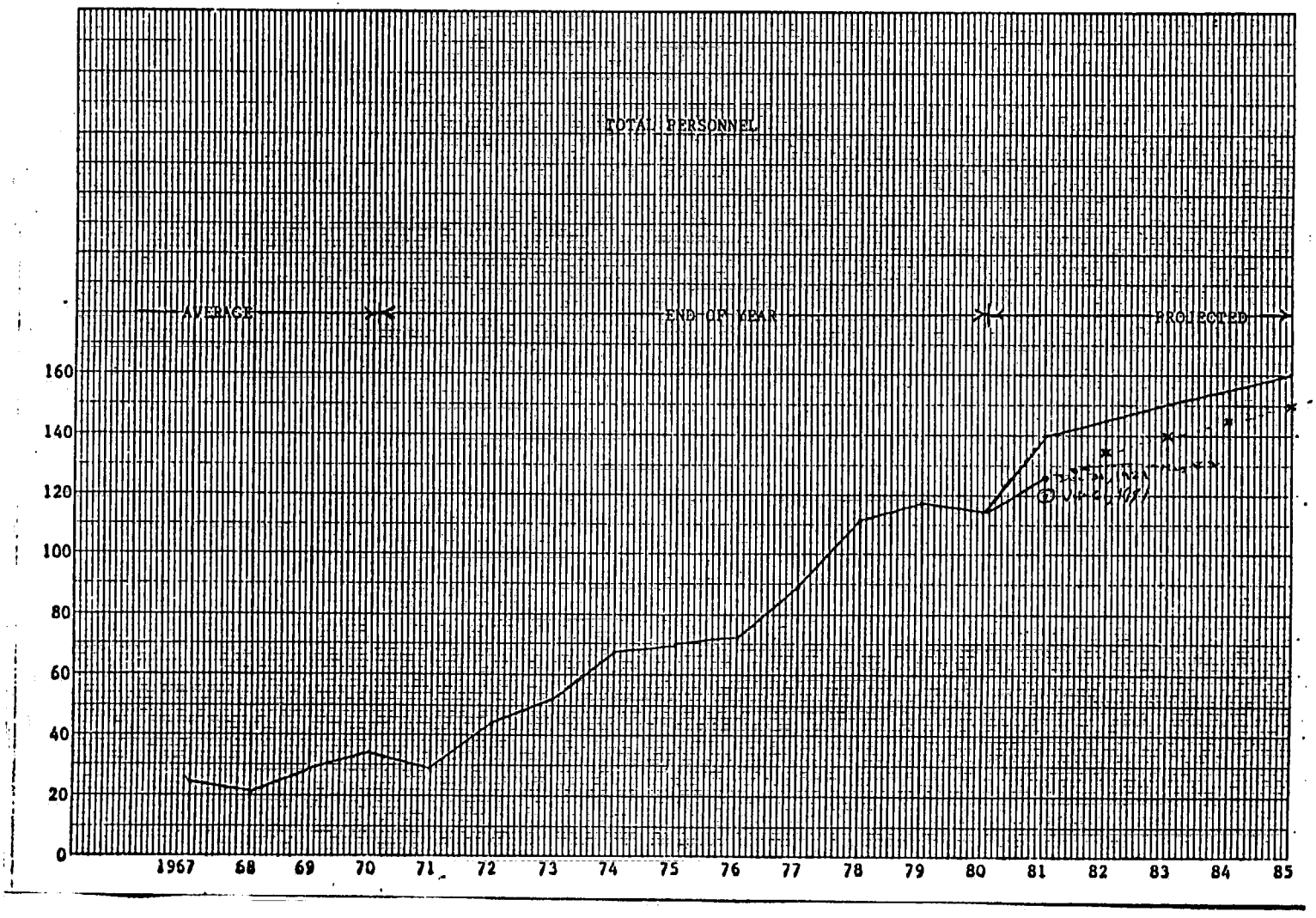

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

VIII. Inhalation Research

1967-1973 Development - smoking machines for inhalation exposure
- inhalation exposure chambers
- animal containment tubes
- INBIFO type SPF inhalation facility

14-Day Inhalation Study - rats - decreased body weight gain.

60-90-Day Inhalation Study - rats and mice
some mortality
less weight gain
hyperplasia and metaplasia in trachea

10-15-Day - Inhalation Study - rat - filter versus non-filer cigarettes
Survival and weight correlate inversely with TPM

CO and nicotine inhalation study - published
CO toxicity - little synergistic effect

1973-1975 2-Year Rat Inhalation Study with expanded tobacco and non-expanded tobacco cigarettes
400-Day exposure + 200-day recovery
No difference between samples except for low incidence of nasal abnormalities
Atrophy of thymus and spleen

1976 90 and 120-Day Inhalation Study on Rats -
Low Delivery Cigarettes - with and without menthol
No difference from 2R1F on equal TPM basis
Both test cigarettes less active than 2R1F
On a per cigarette basis

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Particulate Phase Deposition with DCBP Labeled Smok

42-Day Inhalation Study on Rats

Continuous versus intermittent exposure

Continuous versus intermittent CO exposure

1977 Laryngeal Epithelial Hyperplasia Studies

Varied TPM and gas phase

+ Acetaldehyde

+ NO

+ NO and NO_2

Whole Smoke versus gas phase

2R1, RL Reference, RL Reference + 2% DAP, Low tar Reference - no difference - dose dependent TPM effect

Developed methodology for Walton Type Exposure in rats

7-Day Inhalation Study - Hamster versus rat - NSD Laryngeal Hyperplasia

Developed Methodology for Acute Inhalation Toxicity in Mice

Food Deprivation Study - Thymic and Splenic Atrophy

1978 21-Day Inhalation Study - Rat Net + 2R1 + NO

Laryngeal Hyperplasia and free lung cells - NO decreased number of PMN Leukocytes

14-Day Inhalation Study - Daily Dissection - Acute and Larynx Effects

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Puff exposure with DCBP and 2,4-D Tracers - DCBP preferred

Development of chronic hamster inhalation Methodology

Intermittent exposure, felt collars, method available

42-Day Inhalation - Rat - 2R1, Low Alkaloid, High Activity (LTF IIIA), Low Activity (LTF IIJF) - Dose Dependent TPM Effect on Laryngeal Hyperplasia

21-Day Inhalation Study - Rat - New End Points - in Progress

Serum Glutamic Pyruvate Transaminase

Micronucleus Test - Bone Marrow

Phagocytosis Assay - Macrophage

AHH Induction - Kidney

Plans: Investigate Additional End Points

Histopathology of olfactory epithelium

Goblet Cell Density - Hamster Respiratory Tract

Histochemical Staining of "Free Lung Cells" in situ

Phagocytosis Assay - Hamster Macrophages

Phagocytosis Induced Chemiluminescence

Sister Chromatid Exchange - Resp. Epithelial Cells

Improve AHH Assay

Epoxide Hydratase Assay

Elastase and Collagenase Assay in Macrophages

High Density Aerosols - Paraffin, DOP, Polymers 0.5-2μ

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

IX. Why Mouse Skin Painting?

- Only Universally Accepted Bioassay
  - Carcinogenesis
  - Life Time Study
  - Easily Measured End Points
  - Whole Smoke Condensate
    - Ease of Application
    - Quantitation
    - Correlation with Cigarette Parameters
  - Assay Semi Standardized - Inter Laboratory Comparisons
  - Additive Testing
    - Dr. Wynder and Sword Compound

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

MOUSE SKIN PAINTING

1974	1st Mouse Skin Painting Study
	1R1 - Reproducibility
1976	2nd Mouse Skin Painting Study
	1R1, 2R1, RL Reference, RL Reference + 2% DAP
	Complete Tumorigenesis
	Promotion Assay
1977	DMBA No Effect Level Study
	3rd Mouse Skin Painting Study
	2R1, Low Tar Reference (3 dose levels)
	4th Mouse Skin Painting Study
	2R1, Endotoxin Treatment (immunological stimulation)
1978	5th Mouse Skin Painting Study
	RL Reference, Tobacco Blend, 50% RL/50% Tobacco
	Cocarcinogenesis Study

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

X. NICOTINE PHARMACOLOGY

1977 Nicotine Pharmacology LD_{50}+ED_{50} Mice (convulsions)

Nicotine and Nicotine Derivative Pharmacology ED_{50}

Frog Posture

Nicotine and Derivative/Analogue Pharmacology

Isolated Guinea Pig Ileum (Preganglionic Parasympathetic)

Rat Phrenic Nerve - Diaphragm (Nicotine Relative to Tubocurarine Inhibition)

1978 Nicotine Pharmacology - Blood Pressure - Anesthetized Rat

Pharmacology - Synthetic Compounds

Nicotine and Derivative/Analogue Pharmacology

Isolated Guinea Pig Auricle

Blood Pressure - Rat

Guinea Pig Ileum

Methodology Improvement

Nicotine Pharmacology - Synthetic Compounds (New Series)

Plans: Improve Methodology - Rat BP Assay

Develop Methodology - Guinea Pig Vas Deferens

- Rabbit Aorta Strip

Adrenergic

Nicotine - Receptor Interaction

Structure-Activity Relationship of Various Analogues at Specific Receptor Sites

Example Torpedo Californicus - Electric Fish

Nicotine Metabolism Studies - Radiolabeled Nicotine (Uniform then Specific ^{14}C Labeling)

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

XI. Ames Test at INBIFO

Intact animals required for PM USA work

Separation of animals and microorganisms essential

FTR requires Ames Assay

Additive Testing

Hunter Committee

French Additives List

Process Variables

No Alternative Available in Europe

Example: Hoffmann LaRoche using 3 contract laboratories for testing same compound

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

XII. What Have We Learned?

Inhalation

60, 90 and 120-day inhalation studies yield much the same information as 2-year inhalation study

- Depression of Body Weight Gain
- Thymic and Splenic Atrophy
- Tracheal Hyperplasia
- Mortality
- No Difference between different cigarette types on an equal TPM basis

Thymic and splenic atrophy can be mimiced by food deprivation

90 and 120-Day Inhalation - Rat - Low tar cigarettes with and without menthol - no difference - both cigarettes less active than 2R1F on a per cigarette basis.

Laryngeal Hyperplasia in Rat - Dose dependent TPM effect - cigarettes with differing biological activity have equal activity on an equal dose basis.

Certain flavor release agents have no demonstrable acute inhalation toxicity effects above the background of the reference cigarette. (Guiacol glucoside, poly-(isopropenyl acetate), benzaldehyde-pyrazine release agent).

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New End Points which may be of value

- Serum glutamic pyruvate transaminase - Liver toxicity
- Micronucleus test - *in vivo* mutagenesis - bone marrow
- Phagocytosis-Macrophage - increased activity
- AHH Induction - Kidney - Systemic toxification/detoxification

Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Mouse Skin Painting

100% RL cigarettes have much less complete activity than 2R1

100% RL cigarettes have much less promoting activity than 2R1

Promotion assay increases sensitivity of MSP -

necessary as specific activity decreases and differentiation between low-activity cigarettes becomes more difficult

Cocarcinogenesis assay - logical extension of promotion assay necessary for differentiation of low activity materials such as RL in blends with tobacco - in progress

Low tar standard cigarette testing in progress - more appropriate control for low tar cigarettes. o.K.

Repeated immunological stimulation ~~may~~ does not reduce animal response - in progress

Nicotine Pharmacology - Nicotinic Effects - CNS, cholinergic, adrenergic

Bioassays developed to differentiate effects and establish structure-activity relationships for derivatives and analogues.

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

XIII. The Future?

1. Ames Assay — Sidestream
2. Low Delivery Cigarettes Screening - Inhalation
   - Flavors - Mouse Skin Painting
   - Additives
3. Multiple Nicotine Effects
   - Pharmacological/Metabolic
   - ~~Behavioral~~
4. New Inhalation End Points
   - Broader Spectrum Testing Possible
5. Hunter Type Studies May be Required → Cocoa
6. New Generation of Synthetics?

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Change of Relationship

PM USA Client until 1977 - Munich

Control Led to 5-Year Plan - INBIFO

FTR

More Control in Future - 3-Month Review of Budget

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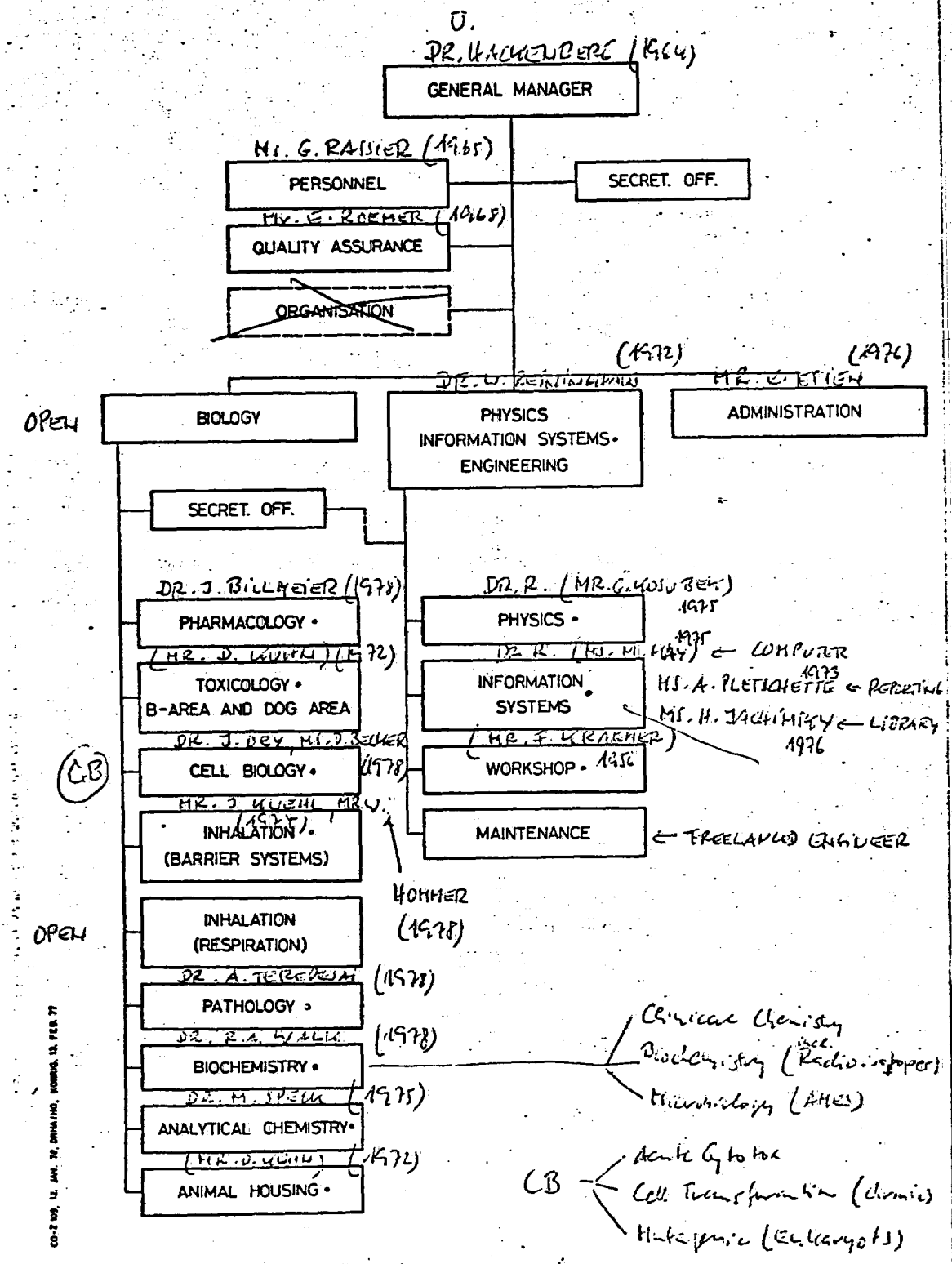
Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000



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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000

Known Third Party Clients

BASF

Bayer

Verband der Cigaretten Industrie

Mercedes?

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Source: https://www.industrydocuments.ucsf.edu/docs/ghml0000